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EXAMINER	
HAMUD, FOZIA M	
ART UNIT	**PAPER NUMBER**
1647	14

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Please find below and/or attached an Office communication concerning this application or proceeding.

Bk copy

Office Action Summary	Application No.	Applicant(s)	
	09/807,558	ANKER ET AL.	
	Examiner	Art Unit	
	Fozia M Hamud	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 May 2003* .

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-31,35-41,46 and 47* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-31,35-41,46 and 47* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

El ction/Restriction

1a. Applicants' responses and arguments filed on 06 February 2003 in Paper No:11 and on 08 May 2003, in Paper No: 13, are acknowledged.

1b. Applicants' response filed on 08 May 2003 has been considered carefully and is found to be persuasive in part. The restriction requirement mailed on 29 November 2002 has been withdrawn. The following restriction requirement is now set forth to clarify the record, to correct errors in previous restriction requirement and to allow Applicants an opportunity to elect anew.

Response to Applicants' Arguments:

2a. Applicants' argument that claims 28, 37, 46 and 47 belong in the same group is persuasive, therefore, these claims are grouped together.

2b. Applicants' argument that the claimed methods are related, as they all use compounds that inhibit SNS activity, is not found persuasive, because the methods recited in the pending claims use products that have no common special technical feature, since a method of administering an agent that reduces sympathetic nervous activity, is not itself an advance over the prior art, because Mueller et al. (Journal of clinical of Investigations, Vol.65, pages 338-346, 1980), describe a method of administering propranolol, (a beta-adrenergic receptor-blocking agent), to patients, and show that propranolol decreases sympathetic nervous activity, (see abstract and page 343, second paragraph).

2c. Applicants' argument that claims 3-27 are species and claims 1, 2, 35, 36 and 41 are generic is not found persuasive, because the products recited in claims 1, 2, 35, 36

and 41, do not fall within the same structural family, nor do these compounds display the same activity.

2d. Applicants' argument that the species election between the diseases recited in claims 29 and 46 is found persuasive, therefore, the requirement to elect a disease species is withdrawn.

Status of Claims:

2a. Claims 1-31, 35-41, 46-47 are pending. Claims 32-34 and 42-45 have already been canceled in the preliminary amendment filed on 17 July 2001 in Paper NO:5).

2b. Applicants' response filed on 06 February 2003 in Paper No:11, was unclear, because the response contained the following statements:

A) "Applicants ***cancel elect*** to prosecute claim 1, as a mended with traverse".

B) and "should the examiner maintain the restriction requirement, please cancel claims 2-43", (see page 5, second paragraph).

Therefore, it was unclear whether Applicants intended to ***cancel or*** to ***elect*** amended claim 1. Also claims 2-43 were never explicitly canceled. Furthermore, claims 32-34 and 42-43 have already been canceled in the preliminary amendment filed on 17 July 2001 in Paper NO:5.

2. In the response filed on 08 May 2003, in Paper No: 13, Applicants stated that in their previous response to the restriction requirement, (i.e the response of 06 February 2003 in Paper No:11), that they have amended claim 1, canceled the remaining claims, and elected to prosecute amended claim 1.

However, neither the response filed on 06 February 2003 in Paper No:11, nor the one filed on 08 May 2003, in Paper No: 13, expressly canceled claims 2-31, 35-41,and 46-47 which are pending in this Application, or clearly elected to prosecute amended claim 1.

In light of all the confusion that exists regarding which claims have been elected and which claims have been cancelled, the Examiner has decided to issue a new restriction requirement, restricting between all the pending claims, (i.e, amended claim 1 and claims 2-31, 35-41, 46-47).

1. This application is a 371 of PCT/GB99/03302. For applications filed under 371, PCT rules for lack of unity apply.

3. This application contains inventions or groups of inventions which are not so linked as to form a single inventive concept. Under PCT Rule 13.1 the following combinations of claims of different categories are permissible and restriction to one of the following combinations is required:

1. Claims 1-3, 19, 29-31, 35-36, 38-39, (in part) and claim 4, drawn to a method of administering to a patient a compound that inhibits the effect of aldosterone.
2. Claims 1-2, 5, 6, 19, 29-31, 35-36, 38-39, 41, (in part) and claim 6, drawn to a method of administering to a patient a chymase inhibitor.
3. Claims 1-2, 7, 19, 29-31, 35-36, 38-39, 41, (in part) and claim 8, drawn to a method of administering to a patient a cathepsin inhibitor.

4. Claims 1-2, 9, 11, 13, 15, 19, 23, 29-31, 35-36, 38-39, 41, (in part) and claims 10, 12, 16 and 24, drawn to a method of administering to a patient a receptor blocker.

5. Claims 1-2, 17, 19, 29-31, 35-36, 38-39, 41, (in part) and claim 18, drawn to a method of administering to a patient a ganglion blocking agent.

6. Claims 1-2, 19, 21, 29-31, 35-36, 38-39, 41, (in part) and claim 20, drawn to a method of administering to a patient an opiate.

7. Claims 1-2, 19, 29-31, 35-36, 38-39, 41, (in part) and claim 22, drawn to a method of administering to a patient a scopolamine.

8. Claims 1-2, 19, 25, 29-31, 35-36, 38-39, 41, (in part) and claim 26, drawn to a method of administering to a patient a xanthine oxidase inhibitor.

9. Claims 1-2, 19, 29-31, 35-36, 38-39, 41, (in part) and claim 27, drawn to a method of administering to a patient an erythropoietin.

10. Claims 1, 19, 29-31, 35-36, 41, (in part) and claim 14, drawn to a method of administering to a patient a receptor agonist.

11. Claims 38 and 39, (in part), drawn to a method of administering to a patient a digitalis alkaloid.

12. Claims 38 and 39, (in part), drawn to a method of administering to a patient a growth hormone.

13. Claims 38 and 39, (in part), drawn to a method of administering to a patient an insulin like growth factor.

14. Claims 38 and 39, (in part), drawn to a method of administering to a patient an endothelin antagonist.

15. Claims 38 and 39, (in part), drawn to a method of administering to a patient a TNF antagonist.

16. Claims 28, 37, 40 and 46-47, drawn to a method of electrically stimulating a patient's muscles.

The inventions listed as Groups 1-16 do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding technical features for the following reasons: The technical feature common to all of the above groups, is a method of administering to a patient an effective amount of an agent which reduces sympathetic nervous activity. However, the method of administering an agent that reduces sympathetic nervous activity, is not itself an advance over the prior art, because Mueller et al. (Journal of clinical of investigations, Vol.65, pages 338-346, 1980), describe a method of administering propranolol, (a beta-adrenergic receptor-blocking agent), to patients, and show that propranolol decreases sympathetic nervous activity, (see abstract and page 343, second paragraph). Because the process of administering an agent that reduces sympathetic nervous activity is not novel, inventions of groups 1-16, do not share a technical feature, therefore, the groups do not relate to a single inventive concept. The invention of Group 1 requires the administration to a patient, an effective amount of a compound that inhibits the effect of aldosterone, which is not required for Groups 2-16.

The invention of Group 2 requires the administration to a patient, an effective amount of a chymase inhibitor, which is not required for Groups 1, 3-16.

The invention of Group 3 requires the administration to a patient, an effective amount of a cathepsin inhibitor, which is not required for Groups 1-2, 4-16.

The invention of Group 4 requires the administration to a patient, an effective amount of a receptor blocker, which is not required for Groups 1-3, 5-16.

The invention of Group 5 requires the administration to a patient, an effective amount of ganglion blocking agent, which is not required for Groups 1-4, 6-16.

The invention of Group 6 requires the administration to a patient, an effective amount of an opiate, which is not required or Groups 1-5, 7-16.

The invention of Group 7 requires the administration to a patient, an effective amount of a scopolamine, which is not required for Groups 1-6, 8-16.

The invention of Group 8 requires the administration to a patient, an effective amount of an xanthine oxidase inhibitor, which is not required for Groups 1-7, 9-16.

The invention of Group 9 requires the administration to a patient, an effective amount of a erythropoietin, which is not required for Groups 1-8, 10-16.

The invention of Group 10 requires the administration to a patient, an effective amount of a receptor agonist, which is not required for Groups 1-9, 11-16.

The invention of Group 11 requires the administration to a patient, an effective amount of a digitalis alkaloid, which is not required for Groups 1-10, 12-16.

The invention of Group 12 requires the administration to a patient, an effective amount of a growth hormone, which is not required for Groups 1-11, 13-16.

The invention of Group 13 requires the administration to a patient, an effective amount of a of insulin like growth factor, which is not required for Groups 1-12, 14-16
The invention of Group 14 requires the administration to a patient, an effective amount of an endothelin antagonist, which is not required for Groups 1-13, 15-16.
The invention of Group 15 requires the administration to a patient, an effective amount of a TNF antagonist, which is not required for Groups 1-14, 16.
The invention of Group 16 requires the electrical stimulation of a patient's muscles, which is not required for Groups 1-15.

The methods of groups 1-16 administer products that do not share a common property or activity. For example, an agent that inhibits the effect of aldosterone, would affect the potassium/sodium exchange in the distal renal tubule; erythropoietin is a glycoprotein that stimulates formation erythroid precursors to generate red blood cells, scopolamine is an anticholinergic agent which acts as a competitive inhibitor at postganglionic muscarinic receptor sites of the parasympathetic nervous system, tumor necrosis factor alpha (TNF alpha) is a critical inflammatory mediator in rheumatoid arthritis. Additionally, the method of Groups 1-16 use products which do not share a common structure. For example, compounds administered in Group I, have the structure of spironolactone, which is a synthetic steroid with an aldosterone-like structure that acts as a competitive antagonist at aldosterone receptors, while compounds administered in Group 2 have the structure of, alendronate , which is a member of the bisphosphonate family of drugs used to treat/prevent osteoporosis. Compounds administered in Group 10, have the structure of clonidine, which is an

imidazoline derivative and exists as a mesomeric compound. Therefore, since no technical feature is shared by these methods, and since the common features do not establish an advance over the prior art, the inventions of Groups 1-16 do not form a single inventive concept within the meaning of PCT Rule 13.2.

4. Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

Claim 1 links inventions 1-10 and claim 38 links inventions 11-15. Depending upon elected Group, the restriction requirement between the linked inventions is subject to the nonallowance of the linking claims, claims 1 or 38. Upon the allowance of the linking claim(s), the restriction requirement as to the linked inventions shall be withdrawn and any claim(s) depending from or otherwise including all the limitations of the allowable linking claim(s) will be entitled to examination in the instant application. Applicant(s) are advised that if any such claim(s) depending from or including all the limitations of the allowable linking claim(s) is/are presented in a continuation or divisional application, the claims of the continuation or divisional application may be

subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application. Where a restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. *In re Ziegler*, 44 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

Having shown that these inventions lack unity for the reasons given above and have acquired a separate status in the art by their recognized divergent subject matter as defined by MPEP § 1850. Therefore, an initial lack of unity for examination purposes as indicated is proper.

Advisory Information:

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Fozia M Hamud whose telephone number is (703) 308-8891. The examiner can normally be reached on Monday, Wednesday-Thursday, 6:30 am to 4:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Kunz can be reached on (703) 308-4623. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Fozia Hamud
Patent Examiner
Art Unit 1647
20 November 2003

Gary L. Kunz

GARY KUNZ
SUPERVISORY PATENT
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Propranolol Decreases Sympathetic Nervous Activity Reflected by Plasma Catecholamines during Evolution of Myocardial Infarction in Man

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ABSTRACT Plasma l-norepinephrine and epinephrine contents were strikingly elevated in 70 patients during evolution of myocardial infarction. Propranolol or placebo, 0.1 mg/kg i.v., was administered randomly an average of 10 h after infarction and continued orally for 3 d. Propranolol, but not placebo, acutely decreased l-norepinephrine contents from 2.24±1.33 (mean±SD) to 1.31±0.74 μg/liter, $P < 0.001$, and epinephrine contents from 0.97±0.42 to 0.74±0.42 μg/liter, $P < 0.02$. Decreases in l-norepinephrine contents were related to the initial plasma concentrations, $r = -0.85$, $P < 0.001$. A similar, but less strong relationship was observed between the initial epinephrine contents and propranolol-induced changes, $r = -0.51$, $P < 0.01$. Propranolol reduced plasma-free fatty acid contents from 1,121±315 to 943±274 μmol/liter, $P < 0.001$. Decreases in plasma contents of free fatty acids were related to decreases in epinephrine, $r = 0.66$, $P < 0.001$. Propranolol did not cause significant additional changes in plasma catecholamine contents during the subsequent 3 d. In the placebo group l-norepinephrine contents had decreased 24 h after infarction from 1.92±0.99 to 1.37 ±0.93 μg/liter, $P < 0.02$. Plasma epinephrine contents did not change. Heart rate remained below the control values during the entire study period in the propranolol, but increased in the placebo group. The data indicate that sympathetic hyperactivity, indirectly reflected by plasma catecholamine contents, is acutely reduced by propranolol during evolution of myocardial infarction.

INTRODUCTION

Plasma catecholamine contents are strikingly increased during evolution and early phase of myocardial infarction. Raab (1) as early as 1943 reported an increase in epinephrine and l-norepinephrine contents during exercise in patients with angina pectoris. Subsequent studies revealed high plasma catecholamine concentrations in myocardial infarction (2, 3) and release of catecholamines locally from ischemic myocardium (4). Serial determinations of plasma catecholamines during early myocardial infarction in man demonstrated that high catecholamine contents correlated with clinical status (5) and hemodynamic findings (6).

Although release of catecholamines initially represents a purposeful response to stress, excess release increases myocardial oxygen consumption and endangers viability of ischemic myocardium. Therefore, beta adrenergic blockade has gained increasing interest as therapeutic intervention in the acute state of myocardial infarction. Propranolol has been shown to be beneficial in experimental coronary occlusion (7) and in human myocardial infarction for a selected patient group (8–10). Recent studies have demonstrated, however, that propranolol increased plasma catecholamine contents in several clinical conditions (11–13), and thus caused concern about its use in acute myocardial infarction. We therefore evaluated the response of plasma catecholamines to propranolol in 35 patients with acute myocardial infarction and compared the results with those obtained after placebo drug. The study demonstrates that in the specific setting of evolving myocardial infarction propranolol acutely decreases plasma catecholamine contents. The complexity of a clinical study does not permit conclusions about mechanisms of action. The almost immediate effect of propranolol could be related to a reduction of afferent sympathetic traffic from ischemic myocardium, or to a direct effect on the central nervous system or on the peripheral sympathetic nerve terminal.

METHODS

Patients admitted to the coronary care unit were considered for the study when the following criteria were met: (*a*) suspected or definite acute myocardial infarction, as evidenced

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by a characteristic history, acute ischemic changes in the electrocardiogram, and, if possible, by plasma creatine kinase MB (CK_{MB}) elevations; (*b*) no electrocardiographic evidence of an old transmural myocardial infarction (Q-waves); (*c*) functional (Killip) (14) classes I and II; (*d*) systolic blood pressure ≥ 95 mm Hg; (*e*) heart rate ≥ 55 beats/min; (*f*) absence of acute bundle branch block, of acute or old second- or third-degree atrioventricular block; (*g*) absence of insulin-dependent diabetes (>20 U/d); (*h*) absence of spastic lung disease; (*i*) age ≤ 75 yr. Informed consent, indicating the randomized, double-blind character of the study, was signed by all patients. The randomization schedule was provided by Ayerst Laboratories, New York.

Experimental procedures. A no. 7 Swan-Ganz thermodilution catheter (Edwards Laboratories, Division of American Supply Corp., Santa Ana, Calif.) was placed into the pulmonary artery. Cardiac output was obtained in triplicate determinations by the thermodilution technique (15). Intravascular and intracardiac pressures were measured with P23d Statham strain gauges (Statham Instruments, Inc., Oxnard, Calif.) and recorded on a multichannel oscilloscope (IM4, Electronics for Medicine, Pleasantville, N. Y.). Blood pressure was obtained by sphygmomanometer.

Each study included measurement of cardiac output, pulmonary artery and capillary wedge pressures, blood pressure, heart rate, substrate analysis of pulmonary artery blood and analysis of oxygen and carbon dioxide tensions and pH of pulmonary artery and arterial blood. After base-line evaluation, 0.1 mg/kg study drug was injected intravenously in three divided doses within 10 min. 20 min after initiation of intravenous injection all measurements were repeated. 40 min after intravenous injection, 40 mg of the study drug was continued per os, increased q6h in 20-mg increments up to 80 mg. Follow-up studies were performed in the fasting state between 6:30 and 8:00 a.m. on the three mornings after admission. These studies were obtained an average of 24, 48, and 72 h after infarction.

Methods of analysis. Plasma epinephrine and l-norepinephrine contents were measured by a modified method of Häggendal (16). The catecholamines were extracted into alumina by batch instead of column adsorption (17), resulting in consistent and increased recovery of 80±6% (SD), $n = 12$, and in minimal oxidation. Dithiothreitol instead of dimercaptopropanol was used as stabilizer, increasing the lutine fluorescence by 57.6% for epinephrine and 50.7% for l-norepinephrine, $n = 10$ (unpublished data). Milli Q2 Millipore water (Millipore Corp., Bedford, Mass.), free from fluorescent impurities was used. The minimum amount detectable with this method is 40 pg for epinephrine and 60 pg for l-norepinephrine. These limits are set by the sensitivity of the spectrophotofluorometer. The precision of the method was evaluated for epinephrine in the range of 0.093–4.34 μg/liter, coefficient of variability (CV),[1] 9.15%, for l-norepinephrine in the range of 0.140–4.24 μg/liter, CV, 9.66%, $n = 28$. Plasma contents, obtained from 10 fasting normal volunteers, 20 min after placement of an intravenous catheter and relaxation in the supine position, averaged 0.146±0.031 (SD) μg/liter (epinephrine) and 0.308±0.071 μg/liter (l-norepinephrine). To evaluate whether propranolol interferes with the analytic method, 100 ng of propranolol hydrochloride (Ayerst Laboratories) was added to 1 ml of plasma obtained from patients in the coronary care unit, but not necessarily with an acute myocardial infarction, $n = 16$. Plasma epinephrine contents averaged 0.703±0.666 μg/liter before, and 0.712±0.671 μg/liter after addition of propranolol, CV, 8.10%; l-norepinephrine contents averaged 1.299±1.121 and 1.292±1.171 μg/liter, respectively, CV, 8.69%. Plasma propranolol contents were determined by a modified method of Shand (18), CV, 3.10%, $n = 20$. Details about determinations of blood concentrations of free fatty acids, of plasma pH, oxygen and carbon dioxide tensions were previously published (8).

The Student's paired *t* test was used for comparisons between adjacent sampling periods. Initial control values were compared to both study periods, 10 min and 3 d after propranolol/placebo administration. All other comparisons were obtained between one sampling period and the period immediately following it (Table I). The *t* test for unpaired data was used to compare results between patients with different infarct locations (Table II).

RESULTS

70 patients with acute myocardial infarction were studied, 35 received propranolol and 35 placebo. Six patients in the propranolol and five in the placebo group were female. The age averaged 57 (41–75) and 56 (39–70) yr in propranolol and placebo groups, respectively. The site of infarction was the anterior or anterior/lateral wall in 15 and 12 patients in the propranolol and placebo groups, the inferior or inferior/posterior wall in 19 and 22 patients, and undetermined in one of each group, respectively. Subendocardial infarctions were observed in two and three instances in each group. Infarct size for the entire patient group averaged 48±33 (SD) CK_{MB}-g-eq (amount of infarcted myocardium liberating CK_{MB} into the circulation equivalent to the amount released from 1 g of homogenously necrotic myocardium). 0.1 mg/kg of study drug was administered intravenously in three divided doses within 10 min, propranolol an average of 10.0 (5.3–13.0) h and placebo an average of 9.6 (4.2–13.2) h after onset of infarction. The study drug was continued per os with an average dose of propranolol of 182±76 (SD) mg during the 1st d of infarction, of 217±111 mg during the 2nd d, and of 214±121 mg during the 3rd d. The corresponding doses for placebo per os averaged 249±33, 304±57, and 293±72 mg, respectively. The plasma propranolol contents immediately after intravenous injection averaged 89±33 (SD) ng/ml, at 24 h after infarction 53±56 ng/ml, at 48 h 162±148 ng/ml, and at 72 h 154±133 ng/ml. There were two in-hospital cardiac deaths in the propranolol group (sudden arrhythmias) and one death in the placebo group (cardiac rupture, verified by autopsy).

Acute study. Plasma l-norepinephrine and epinephrine contents before drug administration were elevated more than fivefold, averaging for l-norepinephrine 2.24 and 1.86 μg/liter in the propranolol and placebo groups and for epinephrine 0.97 and 0.90 μg/liter, respectively (Table I). The plasma l-norepinephrine contents of the individual patients before and 20 min after initiation of intravenous drug administration are shown in Fig. 1. After propranolol

[1] *Abbreviation used in this paper*: CV, coefficient of variability.

TABLE I

Plasma Catecholamine and Substrate Contents and Hemodynamics in Acute Myocardial Infarction

Average hours p acute myocardial infarction	10				24		48		72	
Prop*	Initial control 35		10 min p intravenous drug 35		34		34		34	
Plac										
Measurement		P		P	Mean±SD					P
1-NE,* μg/liter										
Propranolol	2.24±1.33	<0.001†	1.31±0.74	NS	1.39±0.87	NS	1.40±0.98	NS	1.37±0.90	<0.02
Placebo	1.86±0.87	NS	1.92±0.99	<0.02	1.37±0.93	NS	1.37±1.12	NS	1.61±1.02	NS
EPI, μg/liter										
Propranolol	0.97±0.42	<0.02	0.74±0.42	NS	0.65±0.40	NS	0.67±0.49	NS	0.63±0.44	<0.05
Placebo	0.90±0.53	NS	0.88±0.56	NS	0.80±0.67	NS	0.78±0.76	NS	0.81±0.65	NS
FFA, μmol/liter										
Propranolol	1,121±315	<0.001	943±274	NS	933±288	NS	842±268	NS	766±233	<0.001
Placebo	1,087±262	NS	1,046±244	NS	1,035±252	<0.01	870±254	NS	838±213	<0.001
HR, beats/min										
Propranolol	77±16.21	<0.001	67±11.23	NS	66±9.95	<0.05	70±11.79	NS	71±10.89	<0.001
Placebo	73±16.17	NS	74±14.97	NS	78±12.81	<0.01	85±16.32	<0.05	80±16.21	<0.05
AP_s, mm Hg										
Propranolol	132±19.52	<0.05	126±20.25	<0.001	111±16.11	NS	107±13.11	NS	108±14.49	<0.001
Placebo	126±18.59	NS	125±18.53	<0.01	117±16.23	NS	115±13.97	<0.02	110±15.17	<0.001
AP_D, mm Hg										
Propranolol	88±11.58	NS	86±14.33	<0.001	74±11.35	NS	70±10.32	NS	72±10.34	<0.001
Placebo	84±11.76	NS	85±11.89	<0.001	77±11.19	<0.05	73±10.05	NS	73±10.33	<0.001
Prop	29		29		28		27		27	
Plac	34		34		33		32		32	
Measurement					Mean±SD					
CI, liters/min/m²										
Propranolol	2.64±0.60	<0.001	2.06±0.46	<0.02	2.28±0.45	<0.01	2.54±0.51	NS	2.59±0.45	NS
Placebo	2.61±0.57	NS	2.56±0.58	NS	2.54±0.51	<0.001	2.83±0.47	NS	2.87±0.56	<0.01
SVR, dyn-s-cm⁻⁵										
Propranolol	1,479±359	<0.001	1,752±384	<0.001	1,567±458	<0.02	1,362±464	NS	1,254±297	<0.001
Placebo	1,486±292	NS	1,502±332	NS	1,425±353	<0.001	1,265±237	NS	1,234±266	<0.001
PAP_D, mm Hg										
Propranolol	11.27±4.32	NS	12.42±5.50	NS	10.70±4.77	NS	9.33±4.29	NS	10.23±3.52	NS
Placebo	11.27±5.61	NS	10.54±5.63	NS	9.37±5.11	NS	8.79±4.32	NS	8.27±3.75	<0.01

* Prop, propranolol group; Plac, placebo group; 1-NE, 1-norepinephrine; EPI, epinephrine; FFA, free fatty acids; HR, heart rate; AP_s, systolic arterial pressure; AP_D, diastolic arterial pressure; CI, cardiac index; SVR, systemic vascular resistance; PAP_D, diastolic pulmonary artery pressure.

† *P* values are for paired *t* test. Comparisons were obtained between one sampling period and the period immediately following it. Control values were compared with both study periods, 10 min and 3 d after propranolol/placebo administration.

1-norepinephrine contents decreased in 28 of 35 patients, remained essentially unchanged in four, and increased in three patients. The mean value decreased from 2.24 to 1.31 μg/liter ($P < 0.001$). In contrast, placebo drug did not significantly change the mean value. 1-Norepinephrine contents increased in 12 patients, probably related to stress of the procedures, and fell in 7. The propranolol-induced changes in plasma epinephrine contents were less marked but were statistically significant. They decreased in 25 patients and remained essentially unchanged or increased in 10. The mean value fell from 0.97 to 0.74 μg/liter ($P < 0.02$). Plasma epinephrine contents did not change after placebo administration. Decreases in plasma 1-norepinephrine contents were related to plasma concentrations (Fig. 2). The higher the initial 1-norepinephrine contents, the greater the decreases after propranolol, $r = -0.85$, $P < 0.001$. A similar, but less strong relationship was also observed between the initial plasma epinephrine contents and the propranolol-induced changes, $r = -0.51$, $P < 0.01$.

Plasma-free fatty acid contents were elevated in both patient groups before interventions, averaging 1,121 μmol/liter in the propranolol and 1,087 μmol/liter in the placebo groups, respectively (Table I). Propranolol-induced decreases in free fatty acids correlated with decreases in plasma epinephrine contents, $r = 0.66$, $P < 0.001$. None of the correlations described for the propranolol group was observed in the placebo group.

Mean values of hemodynamic data are shown in Table I. Heart rate and cardiac index significantly decreased after propranolol, but not after placebo ad-

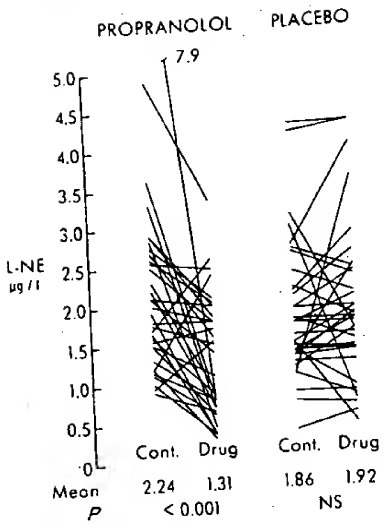

FIGURE 1 Plasma 1-norepinephrine contents of the individual patients before and after intravenous drug administration. After propranolol, 1-norepinephrine contents decreased in 28 patients, remained unchanged in 4 and increased in 3. In contrast, after placebo 1-norepinephrine contents increased in 12 patients, probably a result of the stress of the procedure, and fell in 7.

ministration. Diastolic pulmonary artery pressure remained essentially unchanged in both groups. Systemic vascular resistance significantly increased after intravenous propranolol and remained unchanged after placebo administration. Pulmonary artery oxygen saturation decreased from 72±5.61 to 66±7.03% ($P < 0.001$) in the propranolol and remained unchanged in the placebo group, 71±5.53 and 72±5.29%, respectively.

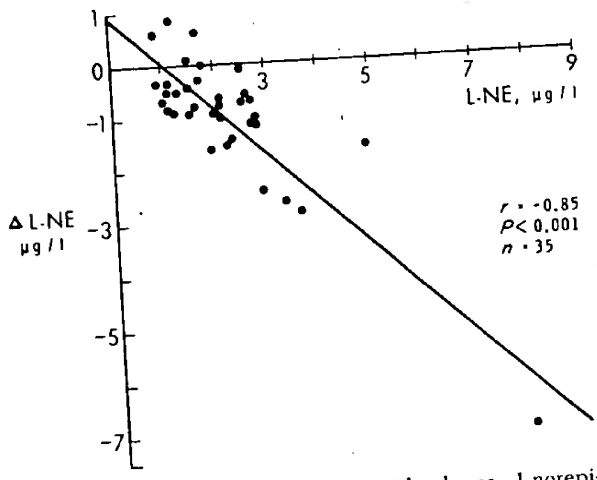

FIGURE 2 Correlation between initial plasma 1-norepinephrine contents and propranolol-induced changes. The higher the initial 1-norepinephrine contents, the greater the decreases after propranolol, $r = -0.85$, $P < 0.001$. 1-NE, 1-norepinephrine.

Sequential studies. Mean values of plasma catecholamine contents and hemodynamic measurements for propranolol and placebo groups are shown in Table I and Fig. 3. The data were obtained an average of 24, 48, and 72 h after onset of infarction. After the acute reduction of plasma 1-norepinephrine and epinephrine contents following intravenous propranolol, there was no significant additional change during the remaining study period. In contrast, intravenous placebo had no acute effect on 1-norepinephrine contents. They decreased during the 1st d from 1.92 (acute study) to 1.37 µg/liter (24 h after infarction). Mean epinephrine contents did not change during the entire study period after placebo injection. The response of plasma-free fatty acids to propranolol was similar to that observed for the catecholamines. After the acute reduction following intravenous propranolol, plasma-free fatty acids remained essentially unchanged during days 1 and 2 and showed a second decrease on day 3; this change was significant when compared with the results of day 1 ($P < 0.01$). In the placebo group plasma-free fatty acids remained elevated at least during the 1st d of infarction. They decreased during the 2nd d, the mean value decreased from an average of 1,035 (24 h) to 870 µmol/liter (48 h), $P < 0.01$.

Mean values of heart rate and cardiac index—acutely reduced after intravenous propranolol administration—increased during the 3-d study period. Although heart rate remained below the control values for the entire 72 h, cardiac index had returned close to the initial control measurements at 48 h. In contrast, in the placebo group, heart rate and cardiac index increased during the 3 d of observation and were above the control values at day 3. The trends in systolic and diastolic arterial pressures were similar in both patient groups. All pressure measurements were significantly lower on day 3, compared with the control study. Systemic vascular resistance increased acutely after intravenous propranolol from an average of 1,479 to 1,752 dyn-s-cm^{-5}; the resistance decreased during day 1 and fell below control values at the 72-h study period. In the placebo group, systemic vascular resistance remained essentially unchanged during day 1, and significantly decreased during day 2. There was no significant difference between the systemic vascular resistance of the propranolol and placebo groups during days 2 and 3.

Separation of initial studies according to infarct location. Six patients with inferior/posterior infarctions, who had electrocardiographic evidence of anterior wall subendocardial ischemia, and two patients with undetermined site of infarction were excluded.

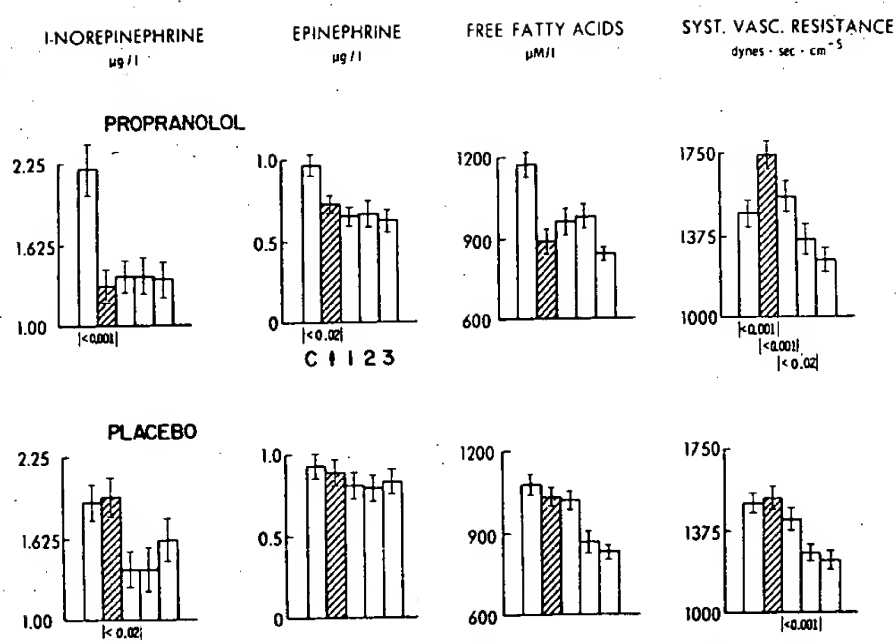

FIGURE 3 Sequential observations of plasma catecholamine and free fatty acid contents and of systemic vascular resistance in propranolol and placebo groups, mean±SE. Propranolol acutely decreased plasma catecholamines and free fatty acids. The effect on systemic vascular resistance was biphasic. In the placebo group, these changes occurred more slowly. Plasma epinephrine did not decrease. C, control; ↑, intravenous drug administration; 1, 2, and 3, days of study after infarction.

Separation of the control data of all study patients before drug administration according to anterior/lateral and inferior/posterior myocardial infarction did not show significant differences between plasma contents of l-norepinephrine, epinephrine and free fatty acids, or between hemodynamic measurements, except for heart rate and pulmonary artery diastolic pressure (Table II). The measurements averaged 84±17 and 69±10 beats/min ($P < 0.001$), and 12±4.15 and 10±4.06 mm Hg ($P < 0.05$), respectively. Grouping of the initial control data according to infarct location separately for propranolol and placebo patients revealed similar results. Changes, induced acutely by intravenous propranolol, did not significantly differ between anterior/lateral and inferior/posterior infarctions, except for the following measurements: heart rate (mean of difference), −15±11 and −7.13±8.75 beats/min ($P < 0.05$); diastolic arterial pressure, −6.13±12 and +2.07±8.34 mm Hg ($P < 0.05$); and cardiac index, −0.72±0.39 and −0.40 ±0.30 liters/min per m² ($P < 0.05$), respectively.

TABLE II

Initial Plasma Catecholamine and Substrate Contents and Hemodynamics in Acute Myocardial Infarction

Infarct location	Anterior/lateral myocardial infarction	Inferior/posterior myocardial infarction	
Measurement	Mean±SD		P
	n = 27	n = 35	
l-NE,* µg/liter	1.95±1.39	2.02±0.86	NS
EPI, µg/liter	0.91±0.33	0.91±0.51	NS
FFA, µmol/liter	1,133±323	1,091±293	NS
HR, beats/min	84±17.12	69±9.78	<0.001
AP_S, mm Hg	130±19.26	128±19.17	NS
AP_D, mm Hg	89±10.29	85±11.97	NS
	n = 25	n = 30	
CI, liters/min/m²	2.58±0.55	2.66±0.56	NS
SVR, dyn-s-cm⁻⁵	1,462±428	1,450±321	NS
PAP_D, mm Hg	12±4.15	10±4.06	<0.05

* Abbreviations: l-NE, l-norepinephrine; EPI, epinephrine; FFA, free fatty acids; HR, heart rate; AP_S, systolic arterial pressure; AP_D, diastolic arterial pressure; CI, cardiac index; SVR, systemic vascular resistance; PAP_D, diastolic pulmonary artery pressure.

DISCUSSION

The increased plasma catecholamine concentrations reported in this and other studies (1–6) reflect increased sympathetic nervous activity, although factors

such as axonal re-uptake, local metabolism within the synaptic cleft, turnover, and binding to receptor sites (19, 20), alter the relationship between neural activity and plasma contents. Sympathetic nervous activity in acute myocardial infarction is influenced by hypotension-mediated baroreceptor reflexes, decreased peripheral perfusion with impaired tissue metabolism (13), cardio-cardiac reflexes (21), anxiety and activation of cardiac vagal afferent fibers (22). The almost immediate decrease of plasma catecholamine contents after administration of propranolol observed in our patients may be the result of a net decrease in afferent sympathetic impulses or a direct effect on the central nervous system or on peripheral sympathetic nerve terminals.

Considerable evidence exists that the release of neurotransmitter from the sympathetic nerve terminal is regulated by a presynaptic feedback system. Stimulation of presynaptic alpha receptors causes negative feedback, decreasing l-norepinephrine release (23–26). Recent studies suggest that positive feedback through stimulation of presynaptic beta receptors also exists that is diminished or abolished by beta adrenergic blockade. Propranolol, but not the dextro isomer, in doses of 0.1 mg/kg, decreased the vasoconstrictor response of the cat hind limb to low-frequency sympathetic nerve stimulation, whereas the response to injected l-norepinephrine was unchanged (27). Propranolol produced similar reductions of constrictor responses to sympathetic nerve stimulation in the guinea pig vas deferens (28), atria (29), oviduct (30), and in human peripheral arteries and veins (25, 31). Isoproterenol increased the overflow of tritiated l-norepinephrine during low-frequency stimulation in the perfused cat spleen; this response was diminished by propranolol, and the reduction was greatest in those experiments with the highest output of neurotransmitter (32, 33). Yamaguchi et al. (26) demonstrated a positive presynaptic feedback control in the open chest dog. Isoproterenol caused a fourfold increase in l-norepinephrine release into the coronary sinus during cardioaccelerator nerve stimulation; this effect was almost abolished by sotalol. Because heart rate, left ventricular dP/dt, and coronary blood flow showed congruent changes after isoproterenol and sotalol administration, the studies suggest that presynaptic beta receptors might play a physiological role in the control of neurotransmitter release.

The experiments, discussed above, indicate that presynaptic beta receptors are sensitive to sympathetic nerve stimulation in the frequency range of 1–10 Hz. Whether these stimulation frequencies are similar to those required for the more than fivefold elevation of l-norepinephrine contents found in our study remains speculative. Observations in man during surgical procedures on the neck (34) have shown that the response of adrenergic-innervated structures is near maximal at 8–10 Hz. Stimulation-effector response curves obtained by Yamaguchi et al. (26, 35) in the dog indicate that the catecholamine concentrations, observed in our patients, are compatible with sympathetic nerve impulses in that range. Consequently, it is possible that the almost immediate decrease in plasma l-norepinephrine contents after intravenous administration of propranolol is related to presynaptic beta adrenergic blockade.

Blockade of central beta adrenergic receptors could also produce a decrease in plasma catecholamine contents. The pressor effect of an intracerebroventricular injection of isoproterenol was blocked by a similarly administered dose of propranolol (36). A biphasic effect was observed when propranolol was injected into the cerebral ventricles; an initial hypertensive response was followed by a reduction in blood pressure within 20–30 min (37). These effects could not be demonstrated with the dextro isomer of propranolol which has little beta blocking activity. Intravenous propranolol decreased sympathetic activity recorded from a preganglionic peripheral sympathetic nerve (38).

Because propranolol has been shown to improve myocardial metabolism within 20 min in human myocardial infarction (8), another explanation for the observed effect on plasma catecholamines might be reduction of afferent impulses that initiated and maintained sympathetic hyperactivity. In contrast to our findings, Hansen et al. (12) observed in individuals with stable ischemic heart disease that intravenous propranolol increased plasma l-norepinephrine contents at rest and during exercise. Afferent sympathetic activity arising from altered tissue metabolism appeared to be more important than baroreceptor mechanisms, because venous oxygen saturation was a much better predictor of l-norepinephrine concentrations than blood pressure. In our patients, propranolol produced changes in hemodynamics and mixed venous oxygen saturation similar to those in Hansen's study at rest, but decreased plasma catecholamine contents, suggesting that some additional factors, related to acute myocardial infarction, were influenced by beta adrenergic blockade, thus overcoming peripheral sympathetic afferent signals.

Brown et al. (39) demonstrated in the vagotomized cat an increase in cardiac afferent sympathetic impulses during left coronary artery occlusion. A similar procedure in the cat enhanced preganglionic activity, demonstrating a cardio-cardiac sympathetic reflex (21). Plasma concentrations of l-norepinephrine were increased after left coronary artery ligation; a series of surgical and pharmacologic interventions indicated that the effect was a result of afferent

impulses arising from the infarcted area (40). The possibility that propranolol in our patients decreased afferent sympathetic stimulation from the infarcted myocardium is supported by observations of Uchida and Murao (41). Intravenous injection of 0.5 and 1.0 mg/kg propranolol before coronary ligation in the dog reduced afferent sympathetic activity from the heart. In consequence, our data are compatible with the hypothesis that sympathetic hyperactivity after acute myocardial infarction in man is related to stimulation of cardiac sympathetic afferent fibers.

Activation of cardiac vagal afferents, however, may modify factors leading to increased sympathetic activity. In patients seen within 30 min of acute myocardial infarction, parasympathetic hyperactivity dominated in inferior/posterior, but was also present in anterior/lateral wall infarctions, whereas sympathetic hyperactivity dominated in the latter group (42). Occlusion of the circumflex, to a lesser degree of the left descending coronary artery in the aortosinus denervated dog, activated receptors producing bradycardia, hypotension, and a decrease in renal sympathetic nerve activity (43). The importance of vagal over sympathetic afferent control in the early minutes after coronary occlusion was supported by a relatively small increase in cardiac sympathetic nerve activity in spite of a depressor response (44). The fact that our initial control data do not show significant differences in plasma catecholamines and in most hemodynamic measurements between patients with anterior/lateral and inferior/posterior infarctions suggests that initial autonomic responses are substantially modified by hemodynamic, metabolic, and emotional stimuli later in the course. The greater response of heart rate to intravenous propranolol in anterior/lateral infarctions probably implies that sympathetic tone was higher in this group.

It is unlikely that the acute decrease in plasma l-norepinephrine contents was produced by a membrane-stabilizing action of propranolol on the neuron that would impede depolarization. Nies and Shand (45) have estimated that plasma concentrations two or three times higher than the usual range of therapeutic concentrations, 80–100 ng/ml, would be necessary to produce a membrane-stabilizing effect. Studies in man have confirmed these differences between concentrations necessary to produce beta adrenergic blockade or membrane stabilization (46), indicating that the average level of 89±33 (SD) ng/ml in our patients was not high enough to directly interfere with depolarization.

The initial increases in peripheral vascular resistance in our patients receiving propranolol is a result, in part, of the decrease in cardiac function, but may also be a result of adrenergic dysbalance at the postsynaptic receptors, resulting in relative increase in alpha adrenergic tone. The impact of alpha adrenergic activity on coronary vascular resistance has been demonstrated in experimental myocardial infarction (47–49) and recently in a clinical study. In patients with stable ischemic heart disease, increase in coronary vascular resistance and chest pain, provoked by cold pressure test, were abolished by alpha adrenergic blockade (50). Although the decrease in catecholamine concentrations demonstrated in our study does reduce the amount of alpha adrenergic stimulation, the plasma levels are still several times greater than normal, and the withdrawal of opposing beta adrenergic activity could produce a net constrictor effect.

The binding of propranolol to several beta adrenergic receptors probably explains the previously reported beneficial effects of the agent in acute ischemic heart disease (7–10) and the differences between propranolol- and placebo-treated patients in this study. The sequential observations shown in Table I and Fig. 3 demonstrate that plasma contents of catecholamines and free fatty acids decline more slowly in the placebo group; heart rate and cardiac index became greater than on admission. Propranolol, in contrast, produces a rapid decrease in plasma catecholamine and free fatty acid concentrations, a biphasic effect on peripheral resistance, and a decrease in heart rate and cardiac index. The effect on plasma catecholamine contents appears to be related to the high initial concentrations encountered in these patients, emphasizing that the net effect of a drug is frequently dependent upon the specific clinical situation selected for evaluation.

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Urinary Kallikrein Excretion in Essential and Mineralocorticoid Hypertension

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ABSTRACT Urinary kallikrein excretion has been reported to be decreased in patients with essential hypertension and elevated in patients with primary aldosteronism as a reflection of mineralocorticoid activity. Low renin essential hypertension (LREH) has been postulated to result from excess production of an unknown mineralocorticoid(s). Urinary kallikrein excretion was compared in outpatients with essential hypertension, mineralocorticoid hypertension (primary aldosteronism and 17α-hydroxylase deficiency), and in normal subjects of the same race. No significant difference in urinary kallikrein excretion of patients with LREH vs. normal renin essential hypertension (NREH) was found for either black (4.1±0.4 vs. 4.8 ±0.5 esterase units (EU)/24 h, mean±SE, for 27 LREH and 38 NREH, respectively) or white patients (12.2±2.3 vs. 11.7±1.4 EU/24 h for 13 LREH and 25 NREH, respectively). Urinary kallikrein was decreased in black vs. white hypertensive patients and normal subjects. However, in patients with normal renal function (creatinine clearance ≥80 ml/min) urinary kallikrein was not significantly decreased in either black hypertensive vs. black normal subjects (4.3±0.3 vs. 5.4±0.6 EU/24 h) or in white hypertensive vs. white normal subjects (11.9±1.2 vs. 8.4±0.9 EU/24 h). In contrast, hypertensive patients with mild renal insufficiency (creatinine clearance of 41.8±78.5 ml/min) had reduced ($P < 0.05$) urinary kallikrein (3.3 EU/24 h with creatinine clearance of 63.6±2.0 for 24 black patients and 4.2± 0.7 EU/24 h with creatinine clearance of 67.0±3.5 for 6 white patients). These results suggest that a reduction in urinary kallikrein excretion rate is an early accompaniment of hypertensive renal injury. Urinary kallikrein excretion in response to a 6-d 10-meq sodium diet and a 3-d Florinef (0.5 mg b.i.d.) administration was compared in hypertensive patients with normal renal function vs. race and age-matched normal subjects. Stimulation of urinary kallikrein excretion by Florinef was equal in black and white normal subjects vs. hypertensive patients (black normals = 12.3±2.7 [n = 9], NREH = 11.7±1.8 [n = 10], LREH = 10.9±1.5 [n = 12]; white normals = 21.2±2.9 [n = 11], essential hypertension = 20.9±3.2 [10 NREH, 5 LREH]). Stimulation of urinary kallikrein excretion with low sodium diet was decreased ($P < 0.05$) only in black LREH (black normals = 11.2±2.4 [n = 10], NREH = 10.1±2.7 [n = 10], LREH = 7.4±1.1 [n = 13]; white normals = 19.1 ±2.7 [n = 13], essential hypertension = 17.5±2.3 [nine NREH, four LREH]). However, during low sodium diet, black patients with LREH had evidence for less sodium depletion as manifested by a decreased rise in urinary aldosterone excretion (16.3±2.7 vs. 33.3±6.4 μg/24 h for black normals) and a failure to achieve metabolic balance in 11/13 patients. Thus, the lesser kallikrein stimulation appeared to result from these two factors. Black and white hypertensives with creatinine clearance <80 ml/min had little increase in urinary kallikrein excretion with Florinef or low sodium diet.

5 of 12 patients with primary aldosteronism or 17α-hydroxylase deficiency did not have an elevated urinary kallikrein excretion rate. Mild renal insufficiency may have contributed to this finding in two of these five patients. Nevertheless, this finding illustrates a limitation to the use of urinary kallikrein excretion rate as an index of mineralocorticoid activity. However, it appears that the majority of patients with LREH have no evidence for excess production of an unknown mineralocorticoid. The failure to find a decrease in urinary kallikrein excretion in racially matched patients with essential hypertension and normal renal function questions the postulate of a role of the kallikrein-kinin system in the initiation of essential hypertension.

INTRODUCTION

In 1934 Elliot and Nuzum noted that hypertensive patients had a decrease in urinary kallikrein excretion

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